

AMENDMENT & RESPONSE UNDER 37 C.F.R. § 1.116 - EXPEDITED PROCEDURE  
Serial Number: 09/820,193  
Filing Date: March 28, 2001  
Title: METHODS TO PREPARE AND USE EPIDERMAL STEM CELLS

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### CLEAN VERSION OF PENDING CLAIMS

1. (Amended) A method to prepare isolated mammalian epidermal stem cells, comprising:
  - (a) providing a sample comprising mammalian epidermal stem cells, which sample is contacted with an agent that binds DNA in viable cells and an agent that binds non-viable cells;
  - (b) separating the sample into a first population of cells that are the smallest 30% of the cells in the sample and a second population of cells that are larger than the cells in the first population, wherein the first population comprises a substantially pure population of epidermal stem cells; and
  - (c) isolating a substantially pure population of epidermal stem cells from the first population of epidermal stem cells.
2. (Amended) A method to prepare isolated mammalian epidermal stem cells, comprising:
  - (a) providing a sample comprising mammalian epidermal stem cells, which sample is contacted with a dye with low or no cellular toxicity that binds DNA in viable cells and an agent that binds non-viable cells;
  - (b) separating the sample into a first population of cells that are the smallest 30% of the cells in the sample and a second population of cells that are larger than the cells in the first population, wherein the first population comprises a substantially pure population of epidermal stem cells; and
  - (c) isolating a substantially pure population of epidermal stem cells from the smaller cells.
3. The method of claim 1 or 2 wherein the mammalian cells are murine cells.
4. The method of claim 1 or 2 wherein the mammalian cells are human cells.
5. The method of claim 1 or 2 wherein the mammalian cells are primate cells.

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6. (Amended) The method of claim 1 or 2 wherein the sample comprises mammalian epidermal cells which have been dissociated from non-epidermal cells.
7. (Amended) The method of claim 1 or 2 wherein the agent which binds DNA is a Hoechst dye.
8. The method of claim 7 wherein the dye is Hoechst 33342.
9. (Amended) The method of claim 1 or 2 wherein the agent that binds non-viable cells is propidium iodide.

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10. (Amended) The method of claim 7 wherein the agent that binds non-viable cells is propidium iodide.
11. The method of claim 1 or 2 wherein the separation is performed with a flow cytometer.
12. (Amended) The method of claim 1 or 2 wherein the sample is in a medium which lacks azide.

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13. (Amended) The method of claim 1 or 2 wherein the sample is further contacted with a nuclear-retained label prior to separation.
14. (Amended) The method of claim 1 or 2 wherein the cells in the second have proliferative capacity.
15. Epidermal stem cells isolated by the method of claim 1 or 2.

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16. (Amended) A method to prepare mammalian epidermal stem cells, comprising:

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(a) contacting a population of mammalian epidermal cells comprising epidermal stem cells with an amount of a first agent under conditions effective for viable cells to retain the first agent;

(b) contacting the population of (a) with an amount of a second agent under conditions effective for non-viable cells to retain the second agent; and

(c) separating the population of (b) into a first population of cells which first population represents the smallest 30% of the cells which comprise viable epidermal stem cells and a second population of cells which represents larger cells, which second population does not comprise a substantial portion of epidermal stem cells.

17. The method of claim 16 further comprising isolating the epidermal stem cells.

18. The method of claim 16 wherein the mammalian cells are murine cells.

19. The method of claim 16 wherein the mammalian cells are human cells.

20. The method of claim 16 wherein the mammalian cells are primate cells.

21. (Amended) The method of claim 16 wherein the sample comprises mammalian epidermal cells which have been dissociated from non-epidermal cells.

22. (Amended) The method of claim 16 wherein the first agent is a Hoechst dye.

23. The method of claim 22 wherein the dye is Hoechst 33342.

24. The method of claim 16 wherein the second agent is propidium iodide.

25. (Amended) The method of claim 16 wherein the sample is further contacted with a nuclear-retained label prior to separation.

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31 26. (Amended) The method of claim 16 wherein the cells in the second population have proliferative capacity.

27. Isolated epidermal stem cells obtained by the method of claim 16.

[NON-ELECTED CLAIMS 28-38 HAVE BEEN WITHDRAWN FROM CONSIDERATION]

39. (Amended) A method to prepare a substantially pure population of mammalian epidermal stem cells, comprising: separating a sample comprising mammalian epidermal stem cells and epidermal cells which are not epidermal stem cells, which sample is contacted with a Hoechst dye and propidium iodine or pyronidine iodine, into a substantially pure population of epidermal stem cells and into at least one population of cells that does not comprise a substantial portion of epidermal stem cells.

40. (Amended) A method to prepare mammalian epidermal stem cells, comprising: separating a sample comprising mammalian epidermal stem cells and epidermal cells which are not epidermal stem cells, which sample is contacted with a Hoechst dye and propidium iodine or pyronidine iodine, into a population which represents the smallest 30% of the cells in the sample and into a population which comprises larger cells, wherein the population with the smallest 30% of the cells comprises a substantially pure population of epidermal stem cells.